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Spectrum of β Thalassemia Mutations and Their Linkage to β-Globin Gene Haplotypes in the Indo-Mauritians

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The β thalassemia alleles in 53 thalassemic Indo-Mauritian patients and their families consisting of 23 homozygous β-thalassemia, 9 HbE/β-thalassemia, 18 HbS/β-thalassemia, 1 HbD/β-thalassemia, 1 δβ/β-thalassemia and 1 HbH/β-thalassemia from the island of Mauritius were studied. Characterization by polymerase chain reaction-based reverse dot blot hybridization technique revealed that the IVS1-5 (G→C) mutation accounted for 74% of the β thalassemic alleles, while six other mutations occurred at much lower frequencies: HbE codon 26 (G→A); 10.4%, codon 8/9 (+G); 3.5%, codon 30 (AGG→ACG) also called IVSI (-1).G→C; 3.5%, codon 15 (G→A); 3.5%, codon 41/42 (−CTTT); 2.4% and -28 (A→G); 2.4%. Association of these mutations to specific β globin gene sequence framework and haplotype allowed to trace their ancestral link. These data are useful in future molecular screening of the population in view of implementing a thalassemia prevention and control program in Mauritius. Am. J. Hematol. 63:11–15, 2000.

Key words: β thalassemia; haplotype; framework

INTRODUCTION

β-Thalassemia is a highly heterogeneous group of inherited disorders of β globin gene expression. More than 140 different β globin gene mutations have been identified in the world population. There is a high frequency of this disorder among people living in regions where malaria is or has been endemic. Each population has its own characteristic set and frequency of β-thalassemia mutations [for a review see Ref. 1]. Orkin et al. [2] observed that the chromosomal region bearing the mutant alleles are in strong linkage disequilibrium to specific patterns of DNA restriction site polymorphisms called β globin gene cluster haplotypes.

The origin of the Mauritian population is diverse. In a total population of about 1.2 million, 68% are of Asian Indian origin, 30% of African, and 2% of Chinese and European ancestry. β-thalassemia is one of the most prevalent single gene defects and is found at a frequency of 4.8% among Indo-Mauritians and 2.2% among Creoles [3]. So far there is no clinical service specifically devoted to thalassemia, and the follow-up of patients is done by pediatricians. Severe forms of homozygous thalassemia cases require expensive and technically demanding curative (bone marrow transplantation) or paliative (chronic transfusion/chelation) therapies. In addition, the disease and the above mentioned therapeutic procedures cause considerable socio-psychological burden for the affected individuals and their families. Overall, the disease drains much of the health resources in Mauritius. As a developing country, carrier screening, genetic counseling, and the offer of prenatal diagnosis are the appropriate options for Mauritius. A prerequisite for such a program is to define the spectrum of β-thalassemia muta-

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tions in the target population. We have applied a PCR-based reverse dot blot (RDB) and denaturing gradient gel electrophoresis (DGGE) techniques to the study of the molecular basis of β-thalassemia in Mauritius. We report here the characterization of β-thalassemia alleles in 53 patients in whom seven different globin gene mutations are found but one allele [IVS1-5 (G→C)] largely predominates (74.1%) over others.

SUBJECTS AND METHODS

Subjects

A total of 53 unrelated Indo-Mauritian families with at least one index case with severe anaemia was included for this study. All these individuals, with thalassemic features and under regular blood transfusion program, were referred from the four major hospitals of the country.

Methods

Phenotype analysis. Abnormal hemoglobins were detected by standard methods (electrophoresis on cellulose acetate at pH 8.6 and citrate agar at pH 6.4 (to distinguish between HbS and HbD and HbE and HbC). The HbA_2 levels were measured either using a commercial kit (Helena HbA_2 thal kit, Beaumont, TX) or high-performance liquid chromatography (HPLC). DNA was extracted from the peripheral leukocytes by a standard phenol–chloroform extraction method.

Mutation analysis by reverse dot blot hybridization. The biotinylated PCR primers (China 1 and PCO6) used in this study as well as the normal and mutant probe sequences for detecting the FS 8/9 (+G), codon 15 (G→A), codon 17 (A→T), codon 26 (G→A), -28 (A→G), codon 30 (G→C), IVS1-1 (G→T), IVS1-5(G→C), FS 41/42 (-CTTT), FS 71/72 (+A) alleles are those described elsewhere [4,5]. The PCR cycling conditions were denaturation at 94°C for 30 seconds, annealing at 55°C for 30 s and extension at 72°C for 30 s for 30 cycles. The RDB membrane hybridization was carried out at 42°C, and signals were revealed by the streptavidin–horse radish peroxidase conjugate (Boehringer Mannheim, GmbH, Germany) as described before [4,5]. The presence of Indian type 619 bp deletional allele was tested by a PCR-based procedure as previously reported [6].

β Globin gene cluster haplotype analysis. Haplotype assignment by the polymorphic profile of seven restriction sites within the β-globin gene cluster was made using already described PCR-RFLP procedures [7,8]. The studied polymorphic restriction sites are Xmn1-5′ Gγ, *Hin*dIII-Gγ, *Hin*dIII-Aγ, *Hin*cII-Ψβ, *Hin*cII-3′Ψβ, *Ava*II-β, and *Hin*f1-3′β.

Globin gene sequence framework analysis. Linked sequence variations within the structural gene (sequence framework) were examined by denaturant gradient gel electrophoresis (DGGE) as described by Ghanem et al. [9]. Thalassemic mutations, defined by RDB, were further confirmed by nucleotide sequencing using dideoxy chain termination method [10] (sequenase Version 2.0 DNA sequencing kit, US Biochemicals, Cleveland, OH).

TABLE I. Spectrum of β-thalassemia Mutations in Indo-Mauritians

Mutation	Alleles	%
IVS-1-5 (G→C)	63	74.1
HbE: codon 26 (A→G)	9	10.6
Codon 30 (AGG→ACG)	3	3.5
FS 8/9 (+G)	3	3.5
Codon 15 (G→A)	3	3.5
−28 A→G	2	2.4
FS 41/42 (−CTTT)	2	2.4
Total	83	100.0

RESULTS

A total of 85 β-thalassemic alleles have been deciphered from 23 homozygous β-thalassemia, 9 HbE/β-thalassemia, 18 HbS/β-thalassemia, 1 HbD/β-thalassemia, 1 δβ/β-thalassemia, and 1 HbH/β-thalassemia from 53 unrelated Indo-Mauritian families. The δβ-thalassemic allele was defined only by the phenotypic characteristics (high HbF, low HbA_2) of the index case and family members.

In a first step, by RDB, we screened for the common mutations (FS 8/9 (+G), codon 15 (G→A), IVS1-1 (G→T), IVS1-5 (G→C), and FS 41/42 (−CTTT)) described in the Indian subcontinent [11]. Then the thalassemic alleles that remained uncharacterized by this procedure were tested for the 619 bp deletion [6] and subsequently analyzed with other probes in a second strip of RDB (codon 17 (A→T), codon 26 (G→A) codon 28 (A→G), codon 30 (AGG→ACG), and FS 71/72 (+A)) [5]. Seven different mutations were detected as shown in Table I, one mutation IVS1-5 (G→C) accounting for 74% of all thalassemic alleles and one-third of it in homozygous state.

Results of haplotype-thalassemic allele association, determined both by the above mentioned PCR-RFLP procedure and DGGE-based framework analysis, are summarized in Table II. The IVS-1-5 (G→C) mutation is associated with three different haplotypes: A, B, and C but all the three having identical sequence framework namely 3 Asian type (3a). The abnormal and thalassemic hemoglobin variant HbE (codon 26 A→G) is linked to haplotype D and sequence framework 2. The codon 30 (AGG→ACG) mutation is associated with haplotype E while FS 8/9 and codon 15 (G→A) mutations with haplotype F. All these three mutations are linked to framework 1 sequence. The codon 41/42 (−CTTT) mutation is

TABLE II. Linkage Disequilibrium Between β-Thalassemia Mutations and β-Globin Gene Haplotypes and Sequence Framework

	Polymorphic sites									
Haplotype	1	2	3	4	5	6	7	Mutation	Framework	Alleles
A	−	−	−	−	−	−	+	IVS1-5, G→C	3 Asian	26
								FS 41/42 , −CTTT	3 Asian	1
B	+	+	−	+	+	−	+	IVS1-5 G→C	3 Asian	1
C	−	+	+	−	+	−	+	IVS1-5 G→C	3 Asian	1
D	+	+	−	+	+	+	−	Codon 26 A→G	2	4
E	+	+	−	+	+	+	+	Codon 30, AGG→ACG	1	3
F	−	−	−	−	−	+	+	FS 8/9 +G	1	2
								Codon 15 G→A	1	2
								FS 41/42, −CTTT	1	1

Studied polymorphic restriction sites for constructing the haplotypes are: 1 = *Xmn*I 5′ to the Gγ-globin gene; 2 and 3 = *Hin*dIII in the large intron of Gγ- and Aγ-globin genes; 4 and 5 = *Hin*cII 5′ and 3′ to the β-globin gene; 6 = *Ava*II in the large intron of β-globin gene and 7 = *Hin*fI 3′ to the β-globin gene.

associated with two haplotypes: haplotype A and F and in DGGE analysis with 2 different sequence frameworks (1 and 3a). Since this is the first molecular characterization involving Mauritius, we opted to confirm the RDB allele assignment both by family studies (obligate heterozygotes) as well as by direct nucleotide sequencing.

DISCUSSION

As shown in Table I, seven mutations, the IVS1-5 (G→C), HbE, or codon 26 (A→G), codon 30 or (IVSI (−1)) (AGG→ACG), codon 8/9(+G), −28 (A→G), codon 15 (G→A), and codon 41/42 (−CTTT) account for the total number of β-thalassemia alleles in these patients. All these mutations are clustered around exon 1 and exon1/intron 1 junction except codon 41/42 (−CTTT) which is on exon 2. The 619 bp deletional allele is not present in this patient group.

These observations have significant implications for developing prevention and control programs of β-thalassemia in Mauritius. In a single strip of RDB, a simple and cost-effective genetic diagnosis is feasible within a day or two.

It is of interest to note that the spectrum of β-thalassemia mutations in Indo-Mauritians is very similar to that of Central Eastern and Southern regions of India. As in India, the IVS1-5 (G→C) allele is the most common mutation in this Island. The codon 30 or (IVSI (−1)) (AGG→ACG), codon 8/9 (+G), codon 15 (G→A), codon 41/42 (CTTT) mutations are also present in the same region of India [11]. The −28 (A→G) and codon 41/42 (−CTTT) are found in three individuals and all had recognized Chinese admixture in their families.

The origin and distribution of the β-thalassemia mutations in Mauritius can be deduced from the haplotypes and frameworks associated with each mutation. Unambiguous haplotype assignment for thalassemic chromosomes was available only for 41 chromosomes out of a total of 83. In vast majority of cases (26 out of 28) the IVS1-5 (G→C) mutation in Mauritius is linked to haplotype A (− − − − − +), identical to that in Asian Indians [12]. In India IVS1-5 (G→C) was found to be associated with eight different haplotypes and two different sequence frameworks whereas in Indo-Mauritians with 3 different haplotypes but on an identical sequence framework. This mutation was considered as the oldest β-thalassemic allele in India, on the basis of its high haplotype diversity as well its wide distribution in this subcontinent. The size of Indian input in Mauritius is quite limited as compared to the population size of the original population. Yet haplotype diversity, associated with IVS1-5 (G→C) mutation is still observed in Mauritius. This mutation occurring in 46% of the β-thalassemic chromosomes in India, exhibits large variations in terms of its regional distribution within India, the highest frequency (more than 80%) being observed in South India [11]. Among Indo-Mauritians, as in South Indians, this mutation represents 74% of the thalassemic chromosomes. Collectively these data suggest and support the historical records that the major Indian input in Mauritius is from Southern and Central Eastern parts of India [16] and the possibility of a founders effect.

All other observed mutations except codon 41/42 (−CTTT) mutation are found in association with haplotypes similar to those found in β-thalassemic chromosomes carrying these mutations on the Indian subcontinent [12]. Concerning the codon 41/42 (−CTTT) mutation, the associated haplotypes are similar to those observed in Chinese and Thais populations [13,14] and the known Chinese admixture in these families are in favor of Chinese origin of this mutation in Mauritius.

Regarding HbE (codon 26, G→A) haplotype association among Indo-Mauritians it is difficult to distinguish between Eastern Indian or South East Asian gene flow because the mutation-haplotype association is identical in these two regions [15].

TABLE III. β^A and β^{thal} Haplotypes in Indo-Mauritians*

	Polymorphic sites									
Haplotype	1	2	3	4	5	6	7	FW	β^A (%)	β^{thal} (%)
	−	−	−	−	−	+	+	1	11 (19)	5 (13.5)
	+	+	−	+	+	+	+	1	4 (7)	3 (8)
	−	+	+	−	+	+	+	1	3 (5)	–
	+	+	−	+	−	+	+	1	1 (1.5)	–
	−	+	+	−	−	+	+	1	1 (1.5)	–
	−	−	−	−	−	+	−	2	6 (10)	–
	+	+	−	+	+	+	−	2	2 (3.5)	–
	−	+	+	−	+	+	−	2	2 (3.5)	–
	−	+	+	−	−	+	−	2	1 (1.5)	–
	−	−	−	−	−	−	+	3	7 (12)	–
	+	+	−	+	+	−	+	3	3 (5)	–
	−	+	+	−	+	−	+	3	2 (3.5)	–
	−	−	−	−	−	−	+	3a	7 (12)	27 (73)
	+	+	−	+	+	−	+	3a	4 (7)	1 (2.5)
	−	+	+	−	+	−	+	3a	2 (3.5)	1 (2.5)
	+	+	−	+	−	−	+	3a	2 (3.5)	–
Total									58	37

*The polymorphic sites are as described for Table II. FW, sequence framework, β^{thal}, beta thalassaemia.

The frequency of haplotypes associated with β^A- and β-thalassemic chromosomes among Indo-Mauritians are summarized in Table III. Diversity of β^A haplotypes is much higher than β-thalassemic haplotypes (16 versus 6, respectively). Each of the thalassemic haplotype is represented among β^A chromosomes. Overall the predominant β-thalassemic haplotypes are also predominant among β^A chromosomes (excepting haplotype D, see below) as has been observed in India on a regional basis [12]. This suggests that a regionally restricted population from India has been introduced into Mauritius. The present molecular data point it out to be the South and Central Eastern regions of India (Tamil Nadu, Andra Pradesh, Orissa, Bihar, and Bengal) consistent with the previously discussed historical records [16–18]. Furthermore the frequency of the haplotype associated with the commonest mutation in Mauritius, namely IVS1-5 (G→C), is represented more than five times than its counterpart associated with β^A. This is consistent with the notion that thalassemic chromosomes undergo positive selection pressure [1] most likely by malaria [19].

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